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PORTON TECHNICAL PAPER No. 848

THE DETECTION OF ANTICHOLINESTERASE AGENTS USING AN ENZYME INHIBITION REACTION [U]

PART VIII.
DEVELOPMENT OF A PORTABLE AUTOMATIC ALARM (MODEL VIII)

A.M. KINNEAR

CHEMICAL DEFENCE EXPERIMENTAL ESTABLISHMENT

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THE DETECTION OF ANTICHOLINESTERASE AGENTS USING AN ENZYME INHIBITION REACTION

PART VIII. DEVELOPMENT OF A PORTABLE AUTOMATIC ALARM (MODEL VIII)

by

A.M. KINNEAR

SUMMARY

A portable automatic alarm (Model VIII) has been designed for the detection of anticholinesterases. It operates from a 24 volt battery at a power consumption of 12 watts, and, excluding the battery, measures 8 in. x 8 in. x 14 in. and weighs 18 lb.

The alarm utilises the enzyme inhibition reaction, the enzyme being pseudo-cholinesterase obtained from horse serum. Three different reagent solutions are required, and these are used in a continuous flow system with continuous sampling of the suspect atmosphere. A photoelectric monitoring of the colour density of the reaction product activates the alarm system when the colour density is decreased.

In preliminary experiments in the gas chambers the alarm responded to 0.015 µg/l. of GB within fifteen seconds.

It is anticipated that this design of alarm may be applicable in principle to other chemical detection systems employing one or more reagent solutions, and in which detection occurs as the result of a change in colour density of the reaction product.

(Sgd.) T.F. Watkins,
Supt., Chemistry Research Division.

(Sgd.) A.S.G. Hill,
Deputy Director.

AMK/MEF

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A.M. KINNEAR

1. INTRODUCTION

An earlier form of Automatic Alarm (Model VII) for detecting anticholinesterases in the atmosphere has been described previously (1). The enzyme inhibition reaction was used with solutions of horse serum pseudo-cholinesterase, guaiacyl butyrate (substrate) and Brentamine Fast Red B Salt* (colour developer to produce a colour with any liberated guaiacol). Sampling and detection were carried on without interruption using a continuous liquid flow system. The alarm responded to concentrations of GB down to 0.01 µg/l. in 10-20 seconds; it was mains-operated, relatively bulky, and not portable.

The successful use of a continuous liquid flow system to give continuous detection with the enzyme inhibition reaction was sufficiently encouraging to warrant attempts to simplify, and to reduce the size and weight of the alarm in order to produce a portable, battery-operated model. In this development work, it was appreciated that some of the sensitivity of the alarm might have to be sacrificed, provided the time to respond to anticholinesterases was not increased.

The effort of modifying and simplifying the Model VII Alarm which led finally to the development of the portable, battery-operated Model VIII Alarm is described in this report.

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*Stabilised diazotised 1-amino-2-methoxy-4-nitrobenzene.

2. GENERAL DESCRIPTION

A flow diagram of the Model VIII alarm is shown in Fig. 1 and photographs of the alarm are shown in Figs. 2-5. The alarm measures 8 in. x 8 in. x 14 in. and weighs 18 lb. The alarm is operated by a 24 volt battery carried separately from the alarm. The power consumption is 12 watts.

The atmosphere is sampled at 2 - 5 l./min using a vane type pump driven directly by a permanent magnet motor. The pump is also coupled to 500:1 reduction gearbox arranged to drive four peristaltic pumps (Figs. 8 and 9). Three of these, driven at about 6 r.p.m., pump the three reagent solutions (enzyme, substrate and colour developer) at 0.4 ml/min into the reaction tube while the fourth pump, driven slightly under 18 r.p.m., draws the reaction product from the separator annulus to the scanning cell.

The sample of air enters at high velocity into the narrow reaction tube (Figs. 1 and 6) where it immediately encounters a small flow of the enzyme solution which it propels as a film along the tube. It is at this stage that the first reaction of the chemical detection system begins, i.e. the inhibition of the enzyme if there is an anticholinesterase agent in the atmosphere.

After travelling successive short distances along the tube, the enzyme solution meets and blends with similar inflows of the substrate and colour developer, when the second and third reactions of the detection system commence.

The solution containing the final reaction product then enters the separator and collects in the annulus while the air passes on via a spray trap to the air pump. From the separator annulus, the liquid is drawn to the scanning cell, through the fourth peristaltic pump and to waste. To enable the reaction product to reach the scanning cell as quickly as possible, the liquid capacities of the separator, scanning cell, and connecting link are made as small as feasible.

Changes in colour density of the solution in the scanning cell are monitored by means of a light source, a matched pair of counterbalanced photocells and a sensitive meter relay. A small reduction in density suffices to close the alarm circuit. This arrangement dispenses with the need for a balancing circuit required when only one photocell is used and compensates for possible variations in light intensity.

The reaction tube terminates in a smooth "dural" block which comes into close contact with the back of the case and lines up with a hole admitting the air sample. The exhaust of the air pump is arranged to line up with another hole in the back of the case at a point remote from the sample inlet so as not to cause interference with the air sampling. For durability, the reagent containers are made of stainless steel. They can be moved aside to give access to the reagent pumps.

3. DETAILED DESCRIPTIONS

(a) Reaction tube design

Important factors involved in the design of the alarm are the flow rate of the liquid, the sampling rate of the air, the bore and length of the reaction tube, the minimum concentration of vapour to be detected, and the activity of the enzyme solution. For a given air flow, the velocity of the air stream and the liquid transfer rate will increase as the diameter of the tube is decreased. Thus the length of the tube will have to be increased when the diameter is decreased if constant reaction times are to be retained. A long narrow tube should be more effective than a shorter wider tube for absorbing C.W. agents from a contaminated air stream, but the narrow tube will offer a higher impedance to the air stream. With the limited amount of power available in a battery-operated portable alarm a satisfactory air flow can only be obtained by keeping the impedance of the tube within reasonable limits. Thus some compromise must be made regarding the dimensions of the reaction tubes.

As there are many possible variables to be considered, it is clear that mathematical calculation of the optimum tube dimensions would be complicated, and so the tube finally adopted in this model was chosen as a result of experiment.

The constructional details of this reaction tube are shown in Fig. 6.

(b) Separator Design

It is necessary to separate the liquid and the air emerging from the reaction tube by a single and continuous process so that the resultant reaction product may be drawn into the scanning cell in a suitable condition for continuous photoelectric monitoring.

One of the main problems in designing a separator was to ensure that a high proportion of the reaction product did not go to waste as spray. It was found that the most successful designs were those in which the "separated" liquid could be pumped to the scanning cell. Based on this experience, a successful separator (Figs. 1 and 6) was designed. In this design the air stream passed along an inner coaxial tube while the liquid was drawn into the annulus by capillary forces. An appreciably larger diameter is used for the inner coaxial tube compared with that of the reaction tube, so that the air velocity is reduced and the tendency for liquid to be drawn off with the air as spray is minimised.

In optimum conditions, the liquid is pumped from the annulus a little slower than it collects in order to avoid drawing air through the annulus. The slight excess of liquid is allowed to go to waste as spray. If the annulus liquid is pumped away too fast, air bubbles will pass through the scanning cell. A slight uniform axial flow of small bubbles through the cell, however, may be tolerated since they assist mixing and only scatter light away from the photocell. This will not cause false alarms when a decrease in colour density is being detected.

A very small annular clearance facilitates rapid transport of the reaction product to the scanning cell.

This type of separator was fabricated in metal and was made from stainless steel and German silver. The design finally selected could easily be dismantled for cleaning.

4. PREPARATION OF ALARM FOR SERVICE

Fill the reagent bottles, empty the waste receiver and spray trap, and ensure that the bulb in the scanning head is clean.

Connect to a 24-volt D.C. supply and switch on. Ascertain that the reagent pumps are functioning, and allow a few moments for expulsion of air pockets from the reagent feed lines. The solution in the scanning head should now be coloured distinctly pur[illegible].

If the illumination lamp has not been adjusted or if it has been replaced, adjust to give maximum light on the detecting photocell. Then adjust the iris diaphragm until the meter relay needle is in the vertical position. The needle should now remain steady until the enzyme becomes inhibited when it will be deflected to the left to close the alarm circuit.

5. OPERATION AND PERFORMANCE

The response time of the alarm appeared to be governed more by the rate of enzyme inhibition than by the rate of substrate hydrolysis. This was indicated by the fact that the response time decreased with increase in enzyme concentration until a point was reached beyond which no further decrease occurred, while increase in substrate concentration merely increased the colour intensity and did not significantly affect the response time. The response time was unaffected by variation in the concentration of the solution of the colour developer since this reaction is practically instantaneous. A slight excess of colour developer was used to allow for possible losses, for example, by decomposition.

The best results were obtained using the reagents in the following concentrations:

Enzyme Solution:	1,000 A.U.* (horse serum cholinesterase) in 1% aquous borax solution.
Substrate Solution:	0.05 ml guaiacyl butyrate dissolved in 4 ml acetone and diluted to 100 ml with water.
Colour Developer Solution:	0.01 g Brentamine Fast Red B salt dissolved in 100 ml water.

The liquid reagents were used at a rate of approximately 0.4 ml/min. The air flow rate was 4 l./min. The alarm was capable of operating for 6-7 hours without attention.

Laboratory tests on the alarm were made by applying a GB contaminated glass stopper to the air intake for a second and noting the time required for the red signal lamp to light up. The detection times were mostly from 8 to 12 seconds and rarely outside the limits 5 and 15 seconds. It should be noted that the detection time is fixed within these close limits by the parameters of the alarm and is almost independent of GB concentration.

The sensitivity of the alarm was ascertained by operating it in known concentrations of GB in a gas chamber. Responses were obtained with all concentrations down to and including .015 µg/l.

Preliminary experiments with GA and VX indicate that for these two agents detection times may be more than 15 seconds.

It is anticipated that this design of alarm may be applicable in principle to other chemical detection systems employing one or more reagent solutions, and in which detection occurs as the result of a change in colour

*The activity unit (A.U.) is defined as the number of micro litres of CO_2 evolved in 30 min per mg dry enzyme preparation when tested by the Warburg method as modified by Strelitz (2).

density of the reaction product.

7. ACKNOWLEDGEMENTS

W.J. Emmens of the Engineering and Respirator Division gave valuable assistance in designing and constructing models throughout this work.

(Sgd.) T.F. Watkins,
Supt., Chemistry Research Division.

(Sgd.) A.S.G. Hill,
Deputy Director.

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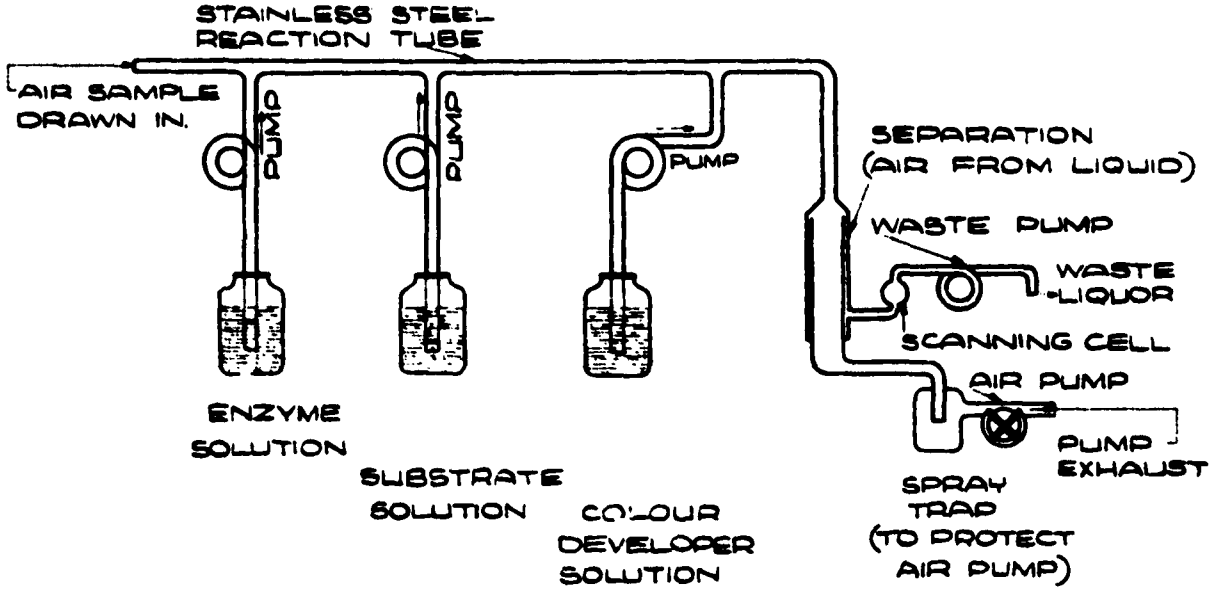


THE AIR IS DRAWN AT 2-5 LITRES/MIN THROUGH REACTION TUBE. REAGENTS PASS AS LIQUID FILM ALONG WALL OF REACTION TUBE AND COLLECT IN ANNULUS IN THE SEPARATOR FROM WHERE THEY ARE PUMPED TO THE SCANNING CELL. THE AIR STREAM BRIDGES THE ANNULUS AND PASSES VIA A SPRAY TRAP TO THE AIR PUMP AND TO WASTE.

DIAGRAM OF MODEL 8 ALARM

FIG 1

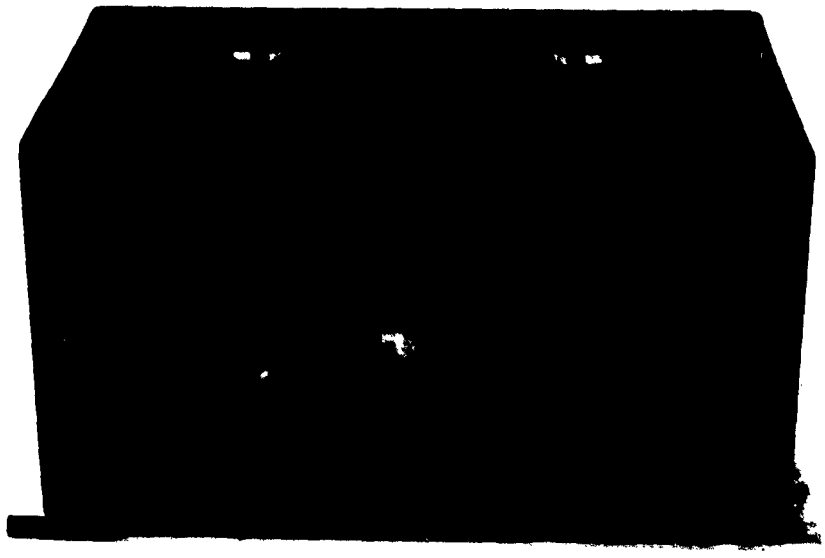


Fig.2. F.896/2

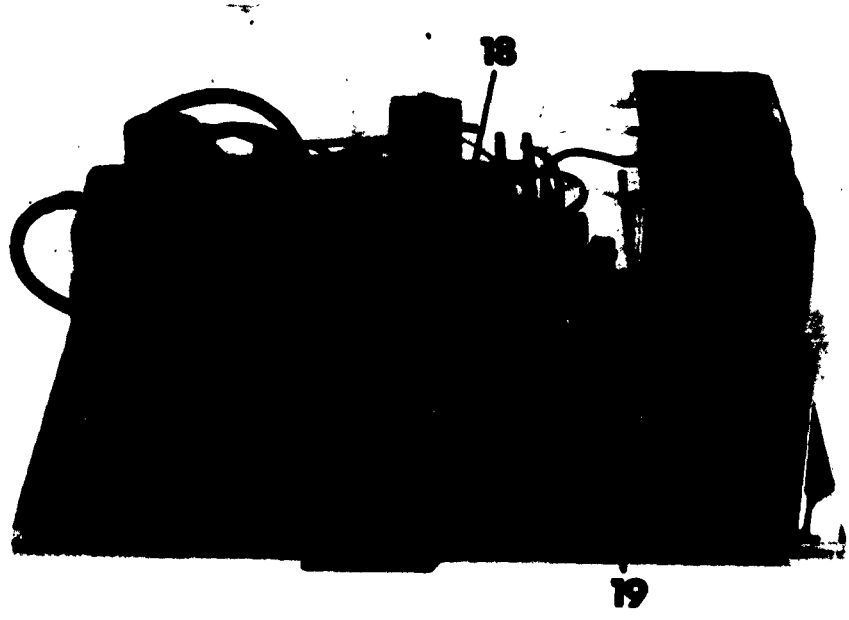
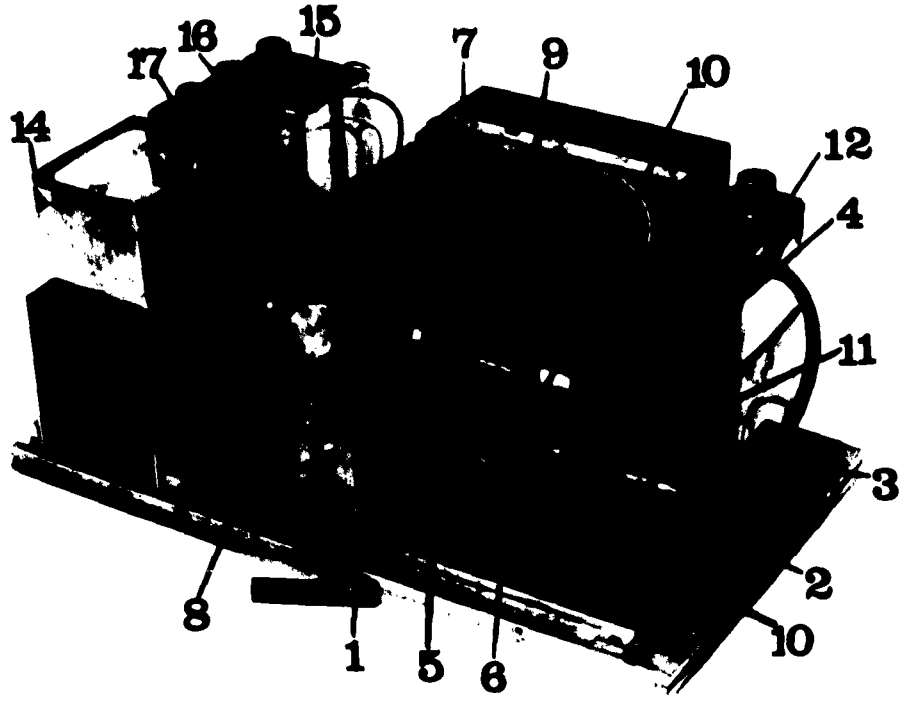


Fig.3. 1705/2

Automatic Alarm Model 7 PTP848



T1705/3

Fig 4

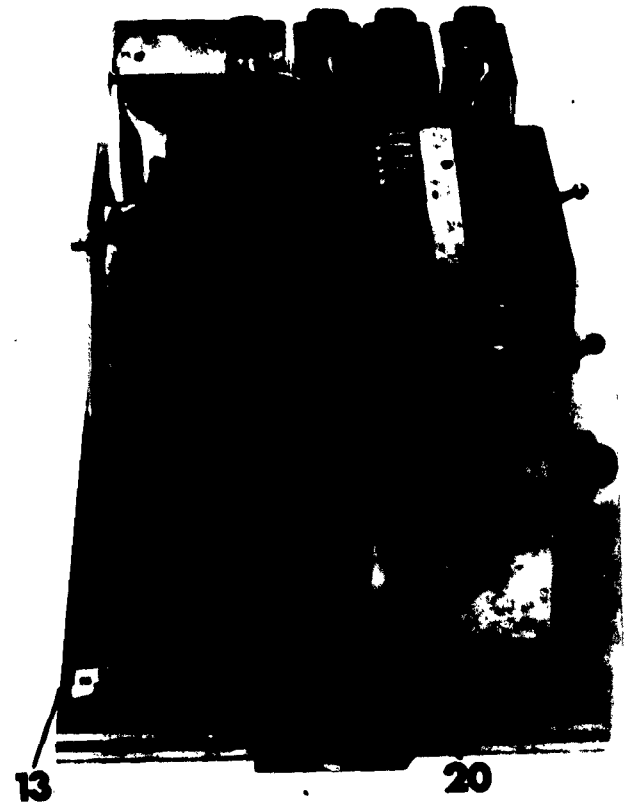


Fig 5
Automatic Alarm Model 7

T1705/1
PTP 848

Key to Figures 3, 4 and 5

1. Reduction gear box.
2. Lamp housing.
3. Iris diaphragm.
4. Compensating photo cell.
5. Detecting photo cell.
6. Glass bulb (for scanning colour of reaction product).
7. Reaction tube.
8. Air intake.
9. Vacuum pump.
10. Liquid-from-Air Separator.
11, 20, 24 volt motor
12. Spray trap
13. Pump exhaust (to hole in case).
14. Stainless steel bottle for waste liquid.
15. Stainless steel bottle for enzyme solution.
16. Stainless steel bottle for colour developer solution.
17. Stainless steel bottle for substrate solution.
18. Main Switch
19. One of the reagent pumps.

DETAILS OF REACTION TUBE - AIR SEPARATOR ASSEMBLY.

FIG.6.

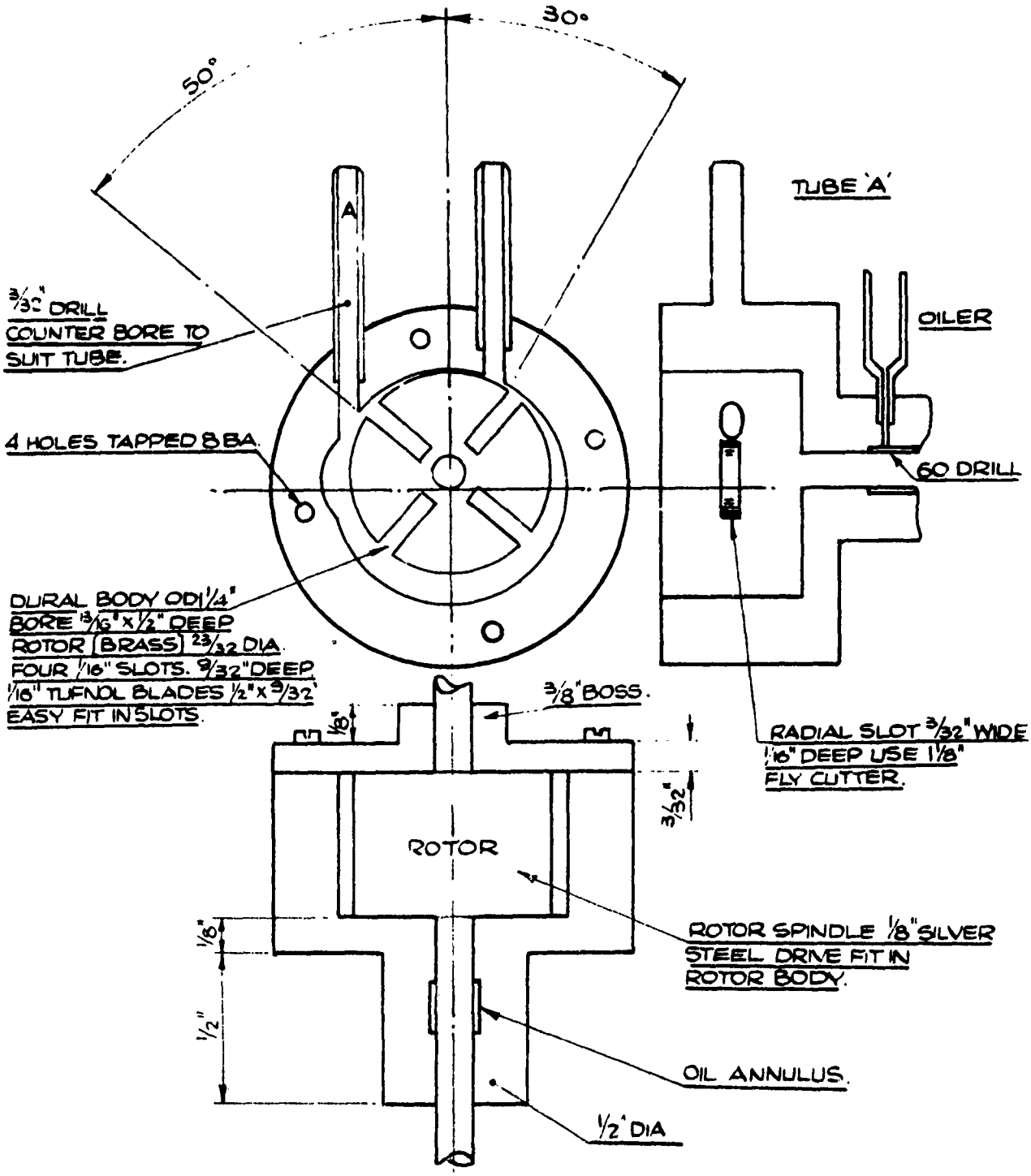


FIG 7 **SUCTION PUMP** SCALE 2/1

P 3078

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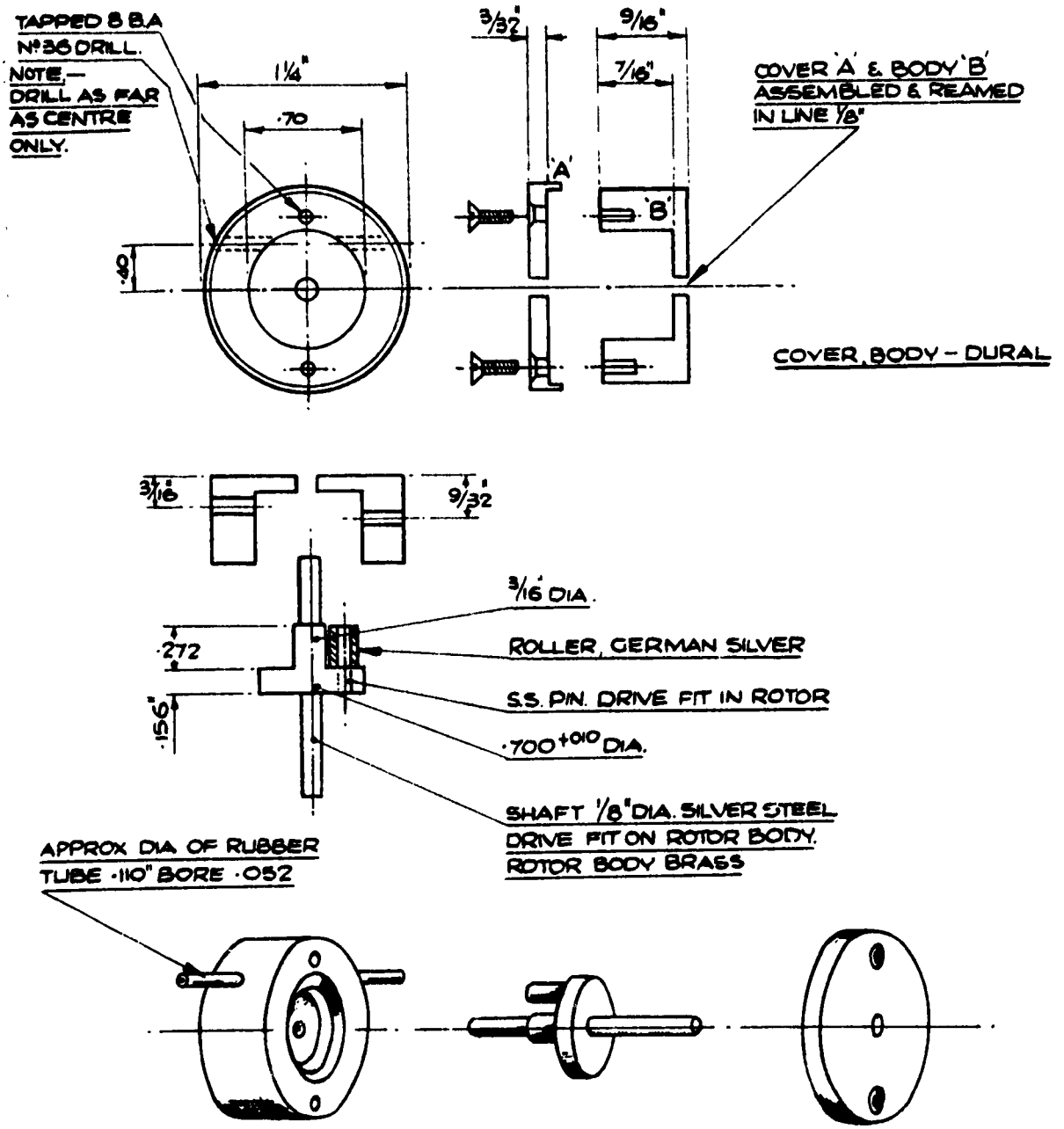

PERISTALTIC PUMP

FIG.8

SCALE 1/1

P 3080

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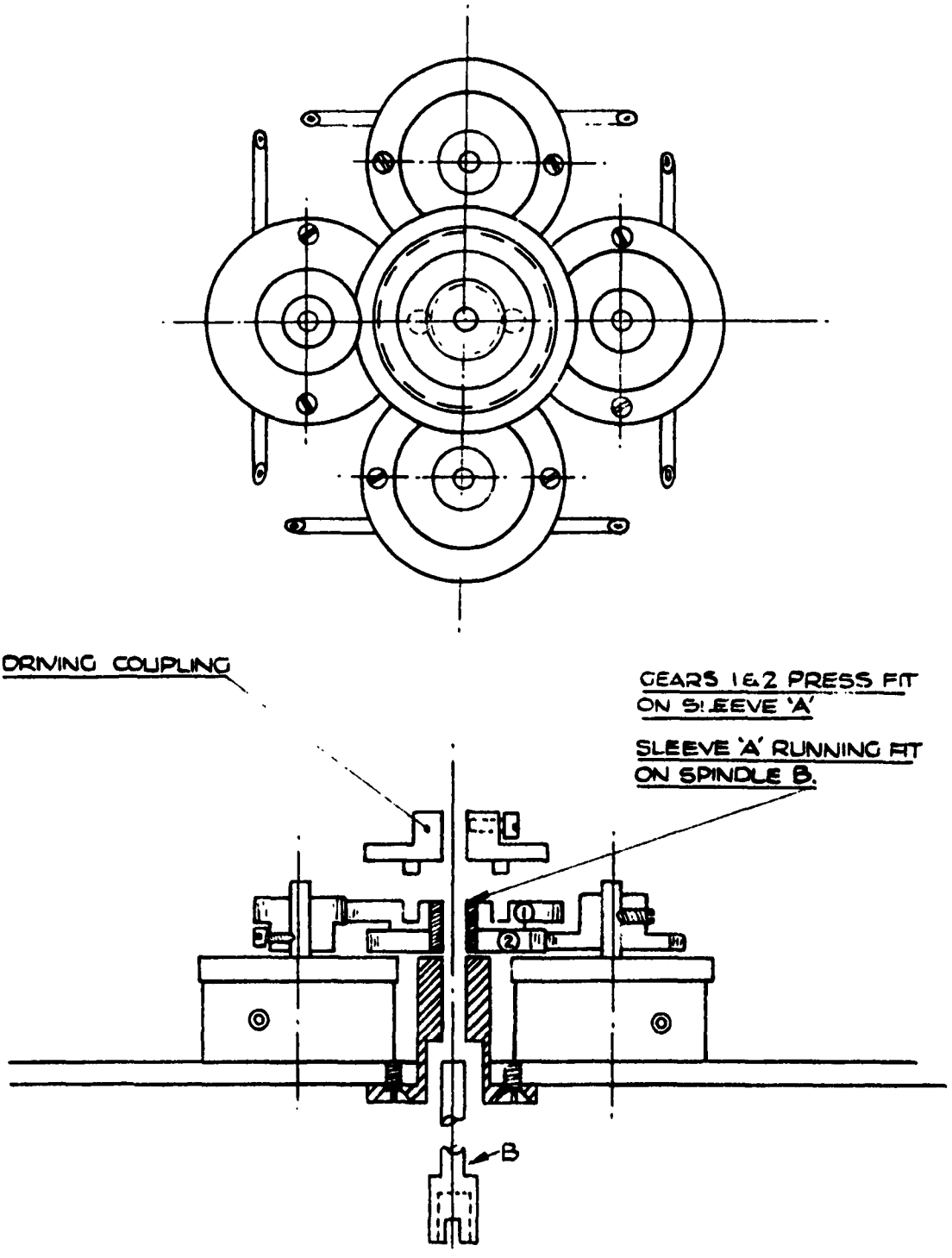

FIG 9 **PUMP & GEAR ASSEMBLY.**

APPROX TO SCALE

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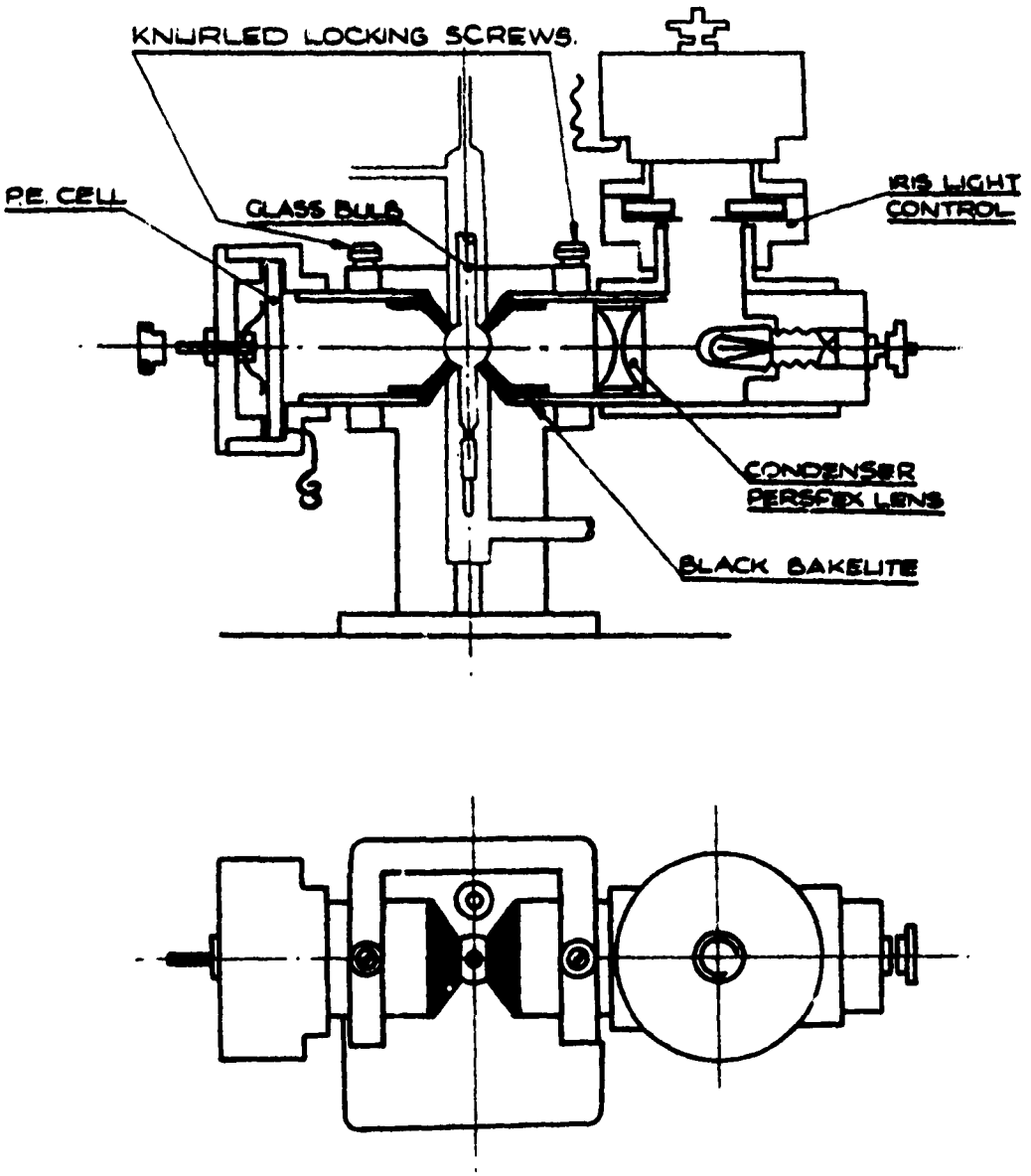
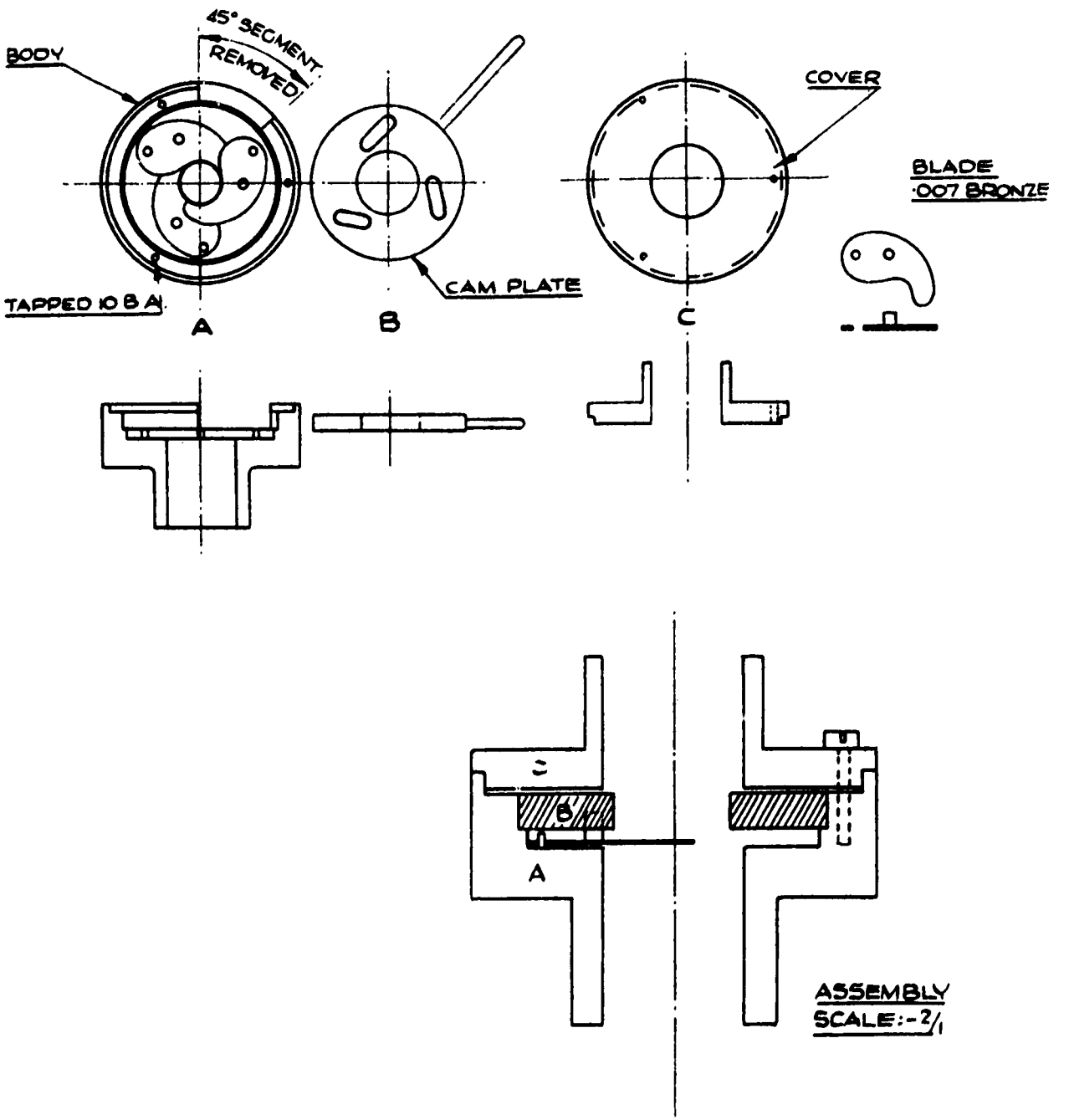
P 3077



SCANNING UNIT

FIG 10



IRIS LIGHT CONTROL

FIG.11

APPROX TO SCALE
BRASS.

P 3079

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PE 1 = DETECTING PHOTO CELL
PE 2 = COMPENSATING PHOTO CELL
B = SCANNING BULB
S = SOCKETS FOR REMOTE CONTROL. RESET SWITCH IF REQUIRED
SW.1 = MAIN ON OFF SWITCH SW.2 = BELL SWITCH
RY.1 = SANGAMO-WESTON SENSITIVE MOVING COIL RELAY TYPE 812A (WITH 24 V RESET.)
RY.2 = MOVING IRON RELAY
DR = DRY RECTIFIER FOR SPARK SUPPRESSION

FIG 12

CIRCIUT DIAGRAM OF MODEL 8 ALARM.

P3081

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